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p-PHENYLAZOANILINE AS AN INSECTICIDE

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During 1940 and 1941 at the Sanford, Fla., laboratory a large number of synthetic organic compounds were tested as insecticides against 16 species of insects. One of the most promising compounds tested was p-phenylazoaniline (p-aminoazobenzene). It was therefore selected for further investigation with regard to its general effectiveness as a spray on truck-crop plants. Most of these investigations were made at Sanford, during 1940 and 1941.

The compound ( $C_6H_5N:NC_6H_4NH_2$ ) is preferably called p-phenylazoaniline, although it is often referred to as p-aminoazobenzene. It is a yellow crystalline solid when pure, but the color of commercial samples may vary from yellow to dark brown. The compound is only slightly soluble in water but is soluble in ether, acetone, and hot ethyl alcohol.

A pure grade of p-phenylazoaniline was used in these tests. The derris standard used in comparative tests contained 4.5 percent of rotenone and the pyrethrum standard 0.33 percent of pyrethrin I and 0.38 percent of pyrethrin II.

p-Phenylazoaniline has been tested on several species of insects by different workers. In 1930 McAllister and Van Leeuwen (5) reported rather poor results with the compound when used as a spray against newly hatched codling moth larvae. Later Fink and coworkers (2) reported the compound toxic to mosquito larvae at 20 parts per million in water, and therefore not particularly promising compared with other compounds reported in the same paper. Bushland (1) used the compound in jar tests with young screw-worm larvae and reported it nontoxic by the method used.

On the basis of the results of Fink and coworkers, application for an insecticide patent was made by D. L. Vivian and H. L. J. Haller (7), of

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<sup>1/</sup> A. M. Phillips was transferred to the Division of Fruit Insect Investigations July 1, 1941.

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this Bureau. This patent describes not only p-phenylazoaniline but also other compounds having two homocyclic nuclei joined by an azo linkage and having one or more amino groups.

The compound was received from the Division of Insecticide Investigations for general testing, as described in a previous publication (5). These tests were designed to obtain preliminary evidence of toxicity, and then to study in more detail the amount of dust or concentration of spray required for satisfactory control of various pests, the utility of the material as a spray on tender foliage, and something of its residual effectiveness when exposed to certain factors of weathering.

### Insects Tested

Since many organic compounds are specific in their toxicity, it was considered that a few tests on a large number of species of insects would be more useful and informative than many replicated tests on a few species. The compound was therefore tested on 16 species representing 4 orders. The majority of the tests were made on leaf-feeding lepidopterous larvae. Nearly full grown larvae were generally used because they are more resistant to insecticides than smaller individuals. The various species used in these tests were as follows:

Insect	Foliage or grain treated
American cockroach ( <u>Periplaneta americana</u> (L.))	None
Cabbage looper ( <u>Autographa brassicae</u> (Riley))	Collards
Cabbage wetworm ( <u>Hellula undalis</u> (F.))	do.
Colorado potato beetle ( <u>Leptinotarsa decemlineata</u> (Say))	Potato
Cowpea weevil ( <u>Callosobruchus maculatus</u> (F.))	Peas (Whippoorwill)
Cross-striped cabbage worm ( <u>Evergestis rimosalis</u> (Guen.))	Collards
Diamondback moth ( <u>Plutella maculipennis</u> (Curt.))	do.
Greenhouse leaf tier ( <u>Phlyctaenia rubigalis</u> (Guen.))	do.
Hawaiian beet wetworm ( <u>Hymenia fascialis</u> (Cram.))	Swiss chard and beet
Imported cabbage worm ( <u>Pieris rapae</u> (L.))	Collards
Melonworm ( <u>Diaphania hyalinata</u> (L.))	Pumpkin
Rice weevil ( <u>Sitophilus oryza</u> (L.))	Wheat
Southern armyworm ( <u>Prodenia eridania</u> (Cram.))	Collards
Southern beet webworm ( <u>Pachyzancla bipunctalis</u> (F.))	Swiss chard and beet
Termites ( <u>Reticulitermes</u> sp.)	None
Yellow woolly bear ( <u>Diaprisia virginica</u> (F.))	Collards

### Preliminary Petri-Dish Tests

Preliminary tests were made in Petri dishes to establish insecticidal action and to determine the species most susceptible to the compound. The tests were made by feeding insects leaf sections that had been rather heavily dusted with the pure compound, as described in a previous publication (6). Dust was applied to both sides of the leaves, and the deposit was determined

approximately by weighing a small metal plate dusted together with the leaves. Two leaves were thus treated for each species and placed in two Petri dishes containing a total of 25 larvae. After 48 or 72 hours an examination was made for mortality of the larvae and an estimate of the amount of feeding. Similar tests were run at the same time on standard insecticides recognized as being effective against the same species of insects.

As the dishes were closed and the larvae able to crawl over the dusted leaves, mortality might result from fumigation, contact, or feeding.

Preliminary tests against 12 species are reported in table 1. The results presented for the cross-striped cabbage worm, the Hawaiian beet webworm, the imported cabbage worm, the melonworm, the southern armyworm, and the southern leet webworm are averages of three replications; those for the other insects represent only one or two tests each. Against four species--the Colorado potato beetle, the cross-striped cabbage worm, the Hawaiian leet webworm, and the melonworm--p-phenylazoaniline was about as effective as the standard insecticide used. In each case a deposit of 70 to 100 micrograms per square centimeter caused either high mortality or very limited feeding. The compound was also toxic to the imported cabbage worm, the southern armyworm, and southern leet webworm but inferior to the standard insecticide. It was highly effective against only one out of the five cabbage insects tested. This apparent resistance of cabbage insects was also evident in the tests made on 1,4-diphenyl semicarbazide (8). As these insects are usually rather susceptible to insecticides, their common resistance to these compounds is of interest from a physiological standpoint.

#### Volatility Tests

The results of the preliminary tests in table 1 establish the fact of toxicity to certain insects but do not give much information on the mode of toxic action. To help clarify this point and aid in the planning of subsequent investigations, the volatility of the compound was determined under certain atmospheric conditions. A glass slide dusted with the powdered chemical was weighed before and after exposure to atmospheric conditions (not sunlight) on a screened porch for 5 days. This procedure showed not more than 3 percent of the deposit volatilized within 5 days, which is ample stability for use as a stomach insecticide on foliage.

It was also evident that the compound was not sufficiently volatile to act as a fumigant. This conclusion was checked, however, by a fumigation test on the melonworm, the cross-striped cabbage worm, and the southern armyworm by isolating some of the powdered chemical in the lids of Petri dishes containing larvae feeding on untreated foliage. These tests verified the conclusion that p-phenylazoaniline did not act as a fumigant in the tests reported in table 1.

Table 1.--Toxicity of p-phenylazoaniline as compared with a standard insecticide when dusted on foliage and fed to nearly full grown larvae of several species confined in Petri dishes

Insect	p-Phenylazoaniline				Standard insecticide			
	Deposit	Kill in		Feeding on	Deposit	Kill in		Feeding on
		2 days	3 days			2 days	3 days	
		Percent	Percent			Percent	Percent	
	Micrograms per sq. cm.				Micrograms per sq. cm.			
Derris								
Cabbage looper	75	43	47	Moderate				
Cabbage webworm	75	2	10	Moderate	170	56		None
	110	28		Trace				
Colorado potato beetle	110	83		Trace	125	100		Trace
Cross-striped cabbage worm	55	73	84	Moderate	55	66	96	Trace
	100	81	96	do.	100	78	96	do.
	155	96	96	Trace	152	78	94	do.
	200	96	100	do.	198	87	96	do.
Diamondback moth	133	18		Moderate	200	100		None
Hawaiian beet webworm	55	54	72	Moderate	55	67	81	Trace
	100	77	88	Trace	100	74	88	do.
	153	76	90	do.	150	77	90	do.
	200	80	97	do.	200	84	96	None
Imported cabbage worm	55	9	45	Moderate	55	88	97	Trace
	100	20	45	do.	100	90	94	do.
	155	37	76	do.	155	93	96	do.
	200	49	75	Trace	200	98	100	do.
Melonworm	55	43	79	Moderate	55	37	71	Trace
	100	63	83	Trace	100	49	80	do.
	150	60	92	do.	155	70	92	do.
	200	73	97	do.	200	70	90	do.
Lead arsenate								
Southern armyworm	55	26	36	Moderate	55	65	96	Trace
	100	46	70	do.	100	84	99	do.
	155	62	89	Trace	155	93	100	do.
	200	66	96	do.	200	97	100	do.
Southern beet webworm	50	7	26	Moderate	50	26	55	Moderate
	100	22	45	do.	100	26	64	do.
	155	33	58	Trace	155	24	71	Trace
	200	35	64	do.	200	53	78	do.
Yellow woolly bear	215	33		Moderate	166	32		Moderate
Pyrethrum								
Greenhouse leaf tier	133	18		Moderate	280	83		Moderate

### Screen-Cage Tests

With the toxicity of *p*-phenylazoaniline established with regard to certain insects, a study was made of the effectiveness of spray deposits on potted plants in cylindrical screen cages. For this purpose the compound was made up as a spray at concentrations of 8, 4, 2, and 1 pound to 100 gallons of water, and each concentration was applied to two plants with a compressed-air spray gun until the spray began to drip from the leaves. When the plants were dry, 15 larvae were confined on each plant by a cylindrical screen cage. The treated plants were then placed out of doors in a sheltered location and examined at two-day intervals for larval mortality and an estimate of the feeding on the plants.

Of the various wetting agents tried, the most satisfactory was saponin. The spray was prepared by grinding the weighed portion of *p*-phenylazoaniline in a mortar with a measured quantity of a saponin solution (equivalent to 1/8 pound of saponin per 100 gallons of spray) and then adding water gradually to obtain the desired concentration. The spray thus formed remained well in suspension, with occasional agitation, and adhered well to foliage.

Spray suspensions of *p*-phenylazoaniline were tested against five species of insects. The results, presented in table 2, are averages of three replicated tests. The compound was very effective against the cross-striped cabbage worm and the melonworm, being about equal to derris, which is very effective on these species. Against the Hawaiian beet webworm *p*-phenylazoaniline appeared to be slightly more effective than derris at all concentrations. It was definitely inferior to lead arsenate against the southern armyworm but slightly superior to this insecticide against the southern beet webworm.

### Phytotoxicity Tests

An experiment was next made to determine the tolerance of certain tender truck-crop plants to spray deposits of *p*-phenylazoaniline. The spray was prepared at concentrations of 4 and 8 pounds per 100 gallons of water, with saponin, and applied to small field plots of bean, collards, pumpkin, and swiss chard. After 10 days a second application was made, and the final results were recorded 20 days after the first application. From 4 to 12 plants of each kind were used with each concentration of spray. The plants were protected from showers and at night to prevent the spray residue from being washed off.

The two applications of the 4-100 spray caused no injury to bean and collards, but they did cause slight injury to pumpkin and swiss chard. A few spot burns were noted on the swiss chard, and moderate to severe burning of the old leaves resulted on pumpkin. The two applications of the 8-100 spray caused no injury to collards and only very slight injury to the old leaves on bean. The injury to swiss chard and pumpkin was similar to that described for the 4-100 spray.

Table 2.--Toxicity of p-phenylazoaniline as compared with a standard insecticide when applied as sprays to potted plants infested with nearly full grown larvae of several insects

Insect	Concentration of insecticide	Tests Pounds per 100 gallons	p-Phenylazoaniline				Standard insecticide			
			Feeding on sixth day	Kill after --			Feeding on sixth day	Kill after --		
				2 days	4 days	6 days		2 days	4 days	6 days
		Number		Percent	Percent	Percent		Percent	Percent	Percent
Derris										
Cross-striped cabbage worm	8	1	Trace	61	97	100	Trace	83	100	100
	4	1	do.	70	93	100	do.	83	100	100
	2	1	do.	58	83	100	do.	68	100	100
	1	1	do.	58	83	86	do.	48	57	96
Hawaiian beet webworm	8	3	Trace	26	79	94	Trace	22	57	69
	4	3	do.	23	67	85	do.	11	51	68
	2	3	do.	7	32	77	do.	6	38	60
	1	3	Moderate	4	34	50	Moderate	2	23	37
Melonworm	8	1	Trace	73	100	100	Trace	30	56	95
	4	1	do.	64	100	100	do.	23	74	98
	2	3	Moderate	26	52	85	do.	11	45	86
	1	3	do.	9	28	58	Moderate	1	18	61
Lead arsenate										
Southern armyworm	8	3	Trace	6	42	49	Trace	71	98	100
	4	3	do.	0	20	46	do.	63	87	99
	2	3	Moderate	2	7	26	do.	60	86	94
	1	3	do.	0	3	24	do.	16	30	52
Southern beet webworm	8	3	Trace	37	78	86	Trace	10	25	49
	4	3	do.	27	57	75	do.	5	22	63
	2	3	Normal	5	13	31	do.	0	3	19
	1	3	do.	2	8	17	Moderate	0	2	10

### Field-Laboratory Tests

A limited study was made of the effect of exposure or weathering on deposits of p-phenylazoaniline on foliage. For this purpose a spray was made up in proportions equivalent to 8 pounds of p-phenylazoaniline and 1/8 pound of saponin to 100 gallons of water and applied with a knapsack-type sprayer to 12 nearly full grown beet plants growing in an outdoor garden. A similar plot was treated with an 8-100 derris (4.5 percent rotenone) spray, and a third plot was left unsprayed as a check. When the plants were dry, six leaf samples (about 2 inches square) were cut at random from each plot and placed in as many Petri dishes for infesting in the laboratory. Five Hawaiian beetle webworm larvae (fifth-instars) were placed in each dish, which was held at room temperature in the laboratory for observation for 72 hours. At the end of 48 and 72 hours the dishes were examined for mortality of the larvae and an estimate of the feeding on the

leaf sections. Similar samples were cut from the plants and fed to larvae in the laboratory every 2 days for 10 days. During this period the sprayed plots were exposed to all weather conditions other than rainfall, from which they were protected by covering.

The results of these tests (table 3) show that a residue of *p*-phenylazoaniline remained effective against the Hawaiian beet webworm for about 4 days on beet plants but lost its effectiveness thereafter. As the compound is not very volatile, this loss in effectiveness would seem to have been caused either by growth of the plant exposing fresh tissue or by a change in the residue through the action of sunlight or other weathering factor. Its effectiveness, however, was equal to that of derris, which caused little mortality after the first 2 or 3 days although causing some repellency throughout the 10-day period.

Table 3.--Results of tests with leaf samples taken at 2-day intervals from beet plants sprayed in the garden with *p*-phenylazoaniline and derris and fed to nearly full grown larvae of the Hawaiian beet webworm in Petri dishes

Time between spraying and sampling	<u><i>p</i>-Phenylazoaniline</u>			<u>Derris (4.5% rotenone)</u>			<u>Check (unsprayed)</u>		
	Feeding	after		Feeding	after		Feeding	after	
		Kill	after -		Kill	after -		Kill	after -
	3 days	2 days	3 days	3 days	2 days	3 days	3 days	2 days	3 days
Days	Percent	Percent		Percent	Percent		Percent	Percent	
0	Trace	30	73	Trace	43	67	Normal	0	0
2	do.	63	83	do.	50	73	do.	6	6
4	do.	10	40	do.	3	10	do.	0	3
6	Moderate	16	33	Moderate	0	3	do.	0	0
8	do.	6	30	do.	6	10	do.	0	0
10	do.	0	13	do.	3	10	do.	0	3

#### Miscellaneous Tests

A preliminary test of *p*-phenylazoaniline was made on the American cockroach by placing 10 nearly full grown nymphs in a 6-inch battery jar on a disk of paper rather heavily dusted with the pure compound. As the paper disk covered the entire bottom area of the jar, the nymphs were certain to come in contact with the powdered chemical. A film of vaseline about the inside upper edge of the jar prevented the escape of the roaches. The jar was kept at room temperature in the laboratory. Under these conditions a deposit of 170 micrograms of dust per square centimeter killed none of the roaches in 3 days. A slightly heavier deposit of sodium fluoride killed 70 percent of the roaches under the same conditions. *p*-Phenylazoaniline was therefore not effective against the American roach, under these conditions at least.

Tests were also made against the cowpea weevil and the rice weevil to find out the possible use of the compound as a grain protectant. A variety called Whippoorwill pea was used with the cowpea weevil, and wheat was used with the rice weevil. The seeds were treated by shaking 15 grams in a flask with a weighed amount of the insecticide until thoroughly mixed. The treated grain was then placed in a Petri dish containing 30 adult weevils and held at room temperature for 3 days. A concentration of 1 part of p-phenylazoaniline to 1,000 parts by weight of peas caused no mortality within 3 days, although in a similar test derris killed 100 percent of the weevils in 2 days. A concentration of 1 part of p-phenylazoaniline to 200 parts of wheat killed none of the rice weevils within 3 days. Derris, in a similar test, killed 36 percent of the weevils. It is apparent that p-phenylazoaniline is not toxic to these weevils under the conditions of the experiment.

The compound was also tested as a soil treatment against termites by a method described by Hockenyos (4). A weighed quantity of the insecticide, according to the concentration desired, and 40 grams of sandy soil were ground in a mortar until thoroughly mixed and then poured into a 150-cc. beaker containing about 12 cc. of water and a little tissue paper. After the dry soil had absorbed the water, 30 adults or large nymphs of the worker caste were placed in the beaker, which was held for 3 days in a cabinet at 80°F. Under these conditions it was found that a concentration of 1-200 of the insecticide in soil killed only 50 percent of the termites in 2 days and a 1-1,000 concentration had practically no effect. Pyrethrum at 1-200 killed 100 percent of the termites within 48 hours.

#### Summary

p-Phenylazoaniline (p-aminoazobenzene) was tested against 12 species of leaf-feeding insects in comparison with standard insecticides generally used with these species. In preliminary tests as a dust on foliage the compound was about as effective as the standard insecticide against the Colorado potato beetle, the cross-striped cabbage worm, the Hawaiian beet webworm, and the melonworm. A volatility test showed the compound to be practically nonvolatile over a period of 5 days, which precludes the possibility of fumigating action.

Spray concentrations of 8, 4, 2, and 1 pound per 100 gallons, with saponin added as a dispersing agent, were applied to potted plants and tested against five species of insects confined by screen cages. In these tests p-phenylazoaniline was equal or superior to the standard insecticide against the cross-striped cabbage worm, the Hawaiian beet webworm, and the melonworm.

Spray concentrations of 8 and 4 pounds per 100 gallons applied to small field plots of tender truck crops caused slight to moderate injury to swiss chard and pumpkin and little or no injury to bean and collards.

Small field plots of beet plants were sprayed with an 8-100 suspension of p-phenylazoaniline and leaf samples taken from the plots at 2-day

intervals for a period of 10 days and fed to Hawaiian beet webworms in the laboratory. The residue was effective for about 4 days but became less effective thereafter.

p-Phenylazoaniline was ineffective against the American roach, the cowpea weevil, the rice weevil, and termites when tested by the methods described.

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